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Epigenetic Modification in Bt and Their Impact on Gene Expression

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Abstract This study explores the epigenetic modifications in *Bacillus thuringiensis* (Bt) and their impact on gene expression. Bt is widely used in biopesticides and genetically modified crops due to its potent insecticidal properties, particularly the production of Cry proteins. However, the increasing issue of resistance in target pests has affected the effectiveness of Bt products. This study investigates the epigenetic regulatory mechanisms of Bt genes, analyzing the roles of DNA methylation, histone modifications, and non-coding RNAs in gene expression regulation. The findings reveal how these epigenetic factors influence the production and efficacy of Bt proteins. The study concludes that epigenetic modifications not only affect Bt's toxicity but may also be key factors in regulating the development of resistance. Therefore, a deeper understanding of these mechanisms can aid in optimizing Bt gene expression, promoting the development of more efficient and sustainable insect-resistant plants, and ultimately reducing reliance on chemical pesticides to ensure environmental safety.

Keywords *Bacillus thuringiensis* (Bt); Epigenetic modifications; Gene expression; Insect resistance; Cry proteins; Crop protection

1 Introduction

Bacillus thuringiensis (Bt) is a Gram-positive bacterium renowned for its insecticidal properties, primarily due to the production of crystal (Cry) proteins. These proteins are highly effective against a variety of insect pests, making Bt a cornerstone in biological pest control and a key component in genetically modified (GM) crops (Gus et al., 2021; Sauka et al., 2023). Bt proteins, such as Cry1Ab and Cry1Ac, have been successfully integrated into crops like chickpea and rice to enhance resistance against pests, thereby reducing the reliance on chemical pesticides and promoting sustainable agriculture (Mehrotra et al., 2011; Liu et al., 2019). The significance of Bt extends beyond pest control, as it also contributes to increased crop yields and reduced environmental impact (Liang et al., 2022).

Epigenetic modifications, including DNA methylation, histone modification, and non-coding RNA interactions, play a crucial role in regulating gene expression without altering the underlying DNA sequence. These modifications can influence the expression of Bt genes in transgenic plants, potentially affecting the efficacy and stability of Bt proteins (Mehrotra et al., 2011). Understanding the epigenetic landscape is essential for optimizing the expression of Bt genes, ensuring consistent pest resistance, and mitigating any unintended effects on plant physiology and development. Moreover, epigenetic mechanisms can provide insights into the long-term sustainability and environmental impact of Bt crops (Liu et al., 2019).

This study explores the epigenetic regulation of Bt genes by identifying key modifications that enhance or suppress gene expression, thereby affecting the production and efficacy of Bt proteins. It investigates the epigenetic modifications associated with Bt genes in transgenic plants and their impact on gene expression, aiming to provide a comprehensive understanding of how epigenetic factors influence the stability and performance of Bt crops, ultimately guiding the development of more efficient and sustainable insect-resistant plants.

2 Understanding Epigenetics in Bt

2.1 Definition and key concepts of epigenetics

Epigenetics is a field that examines heritable changes in gene expression that occur without altering the underlying DNA sequence. These modifications are essential for regulating how genes are activated or silenced, playing a critical role in various biological processes such as development, cellular differentiation, and the organism's response to environmental changes. By influencing gene expression patterns, epigenetic mechanisms help orchestrate complex processes like tissue development and adaptation to external stimuli, thus maintaining cellular and organismal homeostasis (Mathur et al., 2022; Lempiäinen and Garcia, 2023).

Key concepts in epigenetics include DNA methylation, histone modifications, and the roles of non-coding RNAs. DNA methylation involves adding methyl groups to cytosine residues in DNA, often leading to gene silencing and impacting cellular identity and gene regulation. Histone modifications encompass various post-translational changes to histone proteins, such as acetylation and methylation, which can alter chromatin structure and modulate gene expression. Additionally, non-coding RNAs, such as long non-coding RNAs (lncRNAs) and microRNAs, contribute significantly to the regulation of gene expression by interacting with chromatin-modifying complexes and influencing both transcriptional and post-transcriptional processes (Jin et al., 2021; Ji et al., 2023). These mechanisms work together to create a complex and dynamic regulatory network that governs gene expression.

2.2 Mechanisms of epigenetic modifications

Epigenetic modifications involve several key mechanisms that influence gene expression. One fundamental mechanism is DNA methylation, which entails the addition of a methyl group to cytosine residues in DNA. This addition typically leads to gene silencing and is crucial for maintaining cellular identity and regulating gene expression (Davalos and Esteller, 2022; Ji et al., 2023). Another significant mechanism is histone modifications, where histones, the protein components of chromatin, undergo various post-translational modifications such as acetylation, methylation, phosphorylation, and ubiquitination. These modifications can alter the chromatin structure and thereby impact gene expression. For example, histone acetylation generally promotes gene expression by loosening the chromatin structure, whereas histone methylation can either activate or repress gene expression depending on the specific residues modified (Sandholtz et al., 2020; García-Giménez et al., 2021; Zaib et al., 2021).

In addition to these, non-coding RNAs, including long non-coding RNAs (lncRNAs) and microRNAs, also play crucial roles in regulating gene expression. These RNAs can operate at both the transcriptional and post-transcriptional levels. They exert their effects by recruiting chromatin-modifying complexes to specific genomic loci, thus influencing the epigenetic landscape (Kan et al., 2021; Mathur et al., 2022).

2.3 Epigenetic landscape in Bt

In *Bacillus thuringiensis* (Bt), the epigenetic landscape is shaped by similar mechanisms observed in eukaryotic systems, albeit adapted to the prokaryotic context. DNA methylation in Bt can regulate gene expression and is involved in processes such as sporulation and toxin production. Histone-like proteins in Bt may undergo modifications that affect chromatin structure and gene regulation, although the specific types and roles of these modifications are less well-characterized compared to eukaryotes (Jin et al., 2021; Lempiäinen and Garcia, 2023).

Recent studies have highlighted the importance of epigenetic regulation in Bt's ability to adapt to environmental changes and stress conditions. For example, oxidative stress can lead to alterations in the epigenetic landscape, affecting gene expression and potentially contributing to the organism's pathogenicity and resistance mechanisms (García-Giménez et al., 2021). Understanding these epigenetic modifications in Bt can provide insights into its biology and inform strategies for its use in biocontrol and other applications.

3 Types of Epigenetic Modifications in Bt

3.1 DNA methylation in Bt

DNA methylation is a well-studied epigenetic modification that involves the addition of a methyl group to the DNA molecule, typically at the fifth carbon of the cytosine ring, resulting in 5-methylcytosine. This modification can lead to the repression of gene expression by altering the interaction between DNA and transcriptional machinery. DNA methylation is a primary mechanism for epigenetic variation and can induce phenotypic changes in response to environmental stresses (Akhter et al., 2021; Kumar and Mohapatra, 2021).

In plants, for example, DNA methylation plays a significant role in coping with abiotic stresses such as drought, cold, and salinity, and similar mechanisms may be present in Bt (Akhter et al., 2021). The dynamic nature of DNA methylation, regulated by the activities of methyltransferases and demethylases, allows for precise control over gene expression in response to developmental and environmental cues (Kumar and Mohapatra, 2021).

3.2 Histone modifications in Bt

Histone modifications involve the addition or removal of chemical groups to histone proteins, which can influence chromatin structure and gene expression. Common histone modifications include acetylation, methylation, phosphorylation, and ubiquitination. These modifications can either activate or repress gene expression depending on the specific chemical group added and the location of the modification on the histone protein (Neganova et al., 2020; Jin et al., 2021).

For instance, histone acetylation generally leads to an open chromatin structure and active gene transcription, while histone methylation can either activate or repress transcription depending on the context (Neganova et al., 2020). The reversible nature of histone modifications makes them attractive targets for therapeutic interventions, particularly in cancer treatment, where aberrant histone modifications are often observed (Jin et al., 2021).

3.3 Role of non-coding RNAs in Bt

Non-coding RNAs (ncRNAs) are a diverse class of RNA molecules that do not encode proteins but play crucial roles in regulating gene expression at the transcriptional and post-transcriptional levels. NcRNAs include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and small interfering RNAs (siRNAs), among others. These molecules can modulate gene expression by interacting with DNA, RNA, or proteins, thereby influencing chromatin structure, RNA stability, and translation (Huang et al., 2020; Rong et al., 2021; Mathur et al., 2022). For example, ncRNAs can guide DNA methylation and histone modification machinery to specific genomic loci, thereby establishing and maintaining epigenetic states (Mathur et al., 2022). In cancer, ncRNAs have been shown to play significant roles in tumorigenesis and are being explored as potential diagnostic markers and therapeutic targets (Rong et al., 2021).

The epigenetic landscape of Bt is shaped by DNA methylation, histone modifications, and the regulatory actions of non-coding RNAs. These modifications collectively contribute to the dynamic regulation of gene expression, enabling Bt to adapt to various environmental challenges and physiological demands. Understanding these mechanisms in greater detail could provide new insights into the biology of Bt and its applications in biotechnology and agriculture.

4 Mechanisms of Epigenetic Regulation in Bt

4.1 Interaction between DNA methylation and gene expression

DNA methylation is a critical epigenetic mechanism that involves the addition of a methyl group to the fifth carbon of cytosine residues in DNA. This modification can switch gene expression on or off during developmental processes and in response to environmental stresses (Kumar and Mohapatra, 2021). DNA methylation typically occurs at CpG islands in gene promoter regions, leading to transcriptional repression by recruiting methyl-CpG-binding domain proteins and histone modifiers, which propagate inactive epigenetic marks like H3K9me3 (Manna et al., 2023). The dynamic nature of DNA methylation, regulated by writer and eraser proteins, allows for precise control of gene expression, contributing to genome stability and cellular differentiation (Figure 1) (Jin et al., 2021; Gray et al., 2022).

4.2 Role of histone modifications in gene regulation

Histone modifications, such as methylation and acetylation, play a pivotal role in the regulation of gene expression by altering chromatin structure and accessibility. These modifications can either activate or repress transcription depending on the specific chemical groups added to histone tails. For instance, histone acetylation generally promotes gene expression by loosening chromatin structure, whereas histone methylation can have varying effects depending on the specific lysine residue modified (Jin et al., 2021). The interplay between histone modifications and other epigenetic mechanisms, such as DNA methylation and non-coding RNAs, forms a complex regulatory network that fine-tunes gene expression (Kan et al., 2021; Bure et al., 2022).

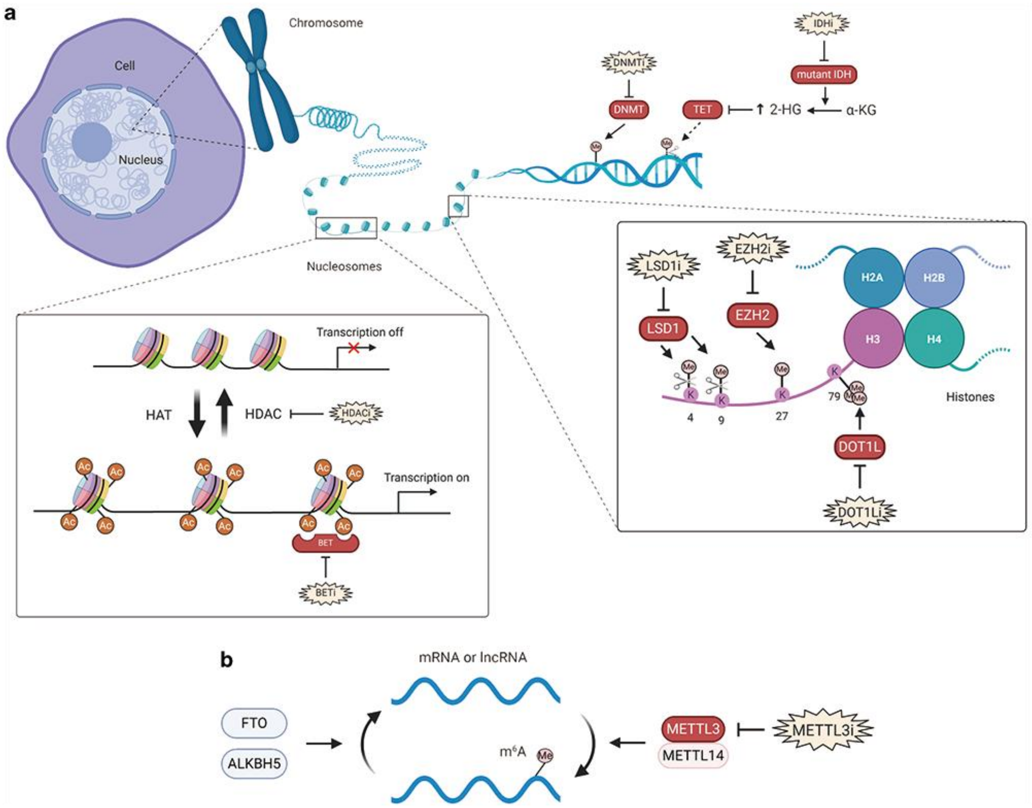

Figure 1 The epigenetic readers, writers and erasers (Adopted from Jin et al., 2021)

Image caption: (a) Histone proteins wrap around DNA to form a nucleosome, which are then compacted to form chromatins and further into chromosomes. (b) A multiprotein complex (consisting METTL3, METTL14 and other subunits) methylates adenosine base at the nitrogen-6 position and forms m^6A in the messenger RNA. The figure illustrates how histone acetylation and methylation affect chromatin structure and gene expression through the action of different enzymes. Histone acetyltransferases (HATs) add acetyl groups, opening the chromatin and promoting gene transcription, while histone deacetylases (HDACs) remove acetyl groups, closing the chromatin and inhibiting transcription. Additionally, methylated histones can either activate or repress gene expression depending on the modification site. The results reveal the dynamic regulation of DNA methylation and RNA modifications, highlighting the complexity and significance of epigenetic mechanisms in gene expression regulation, thus supporting the theoretical basis for epigenetic-targeted therapies (Adapted from Jin et al., 2021)

4.3 Impact of non-coding RNAs on gene expression

Non-coding RNAs (ncRNAs), including microRNAs, piwi-interacting RNAs, and long non-coding RNAs, are crucial regulators of gene expression at the epigenetic level. These ncRNAs can modulate gene expression by guiding chromatin-modifying complexes to specific genomic loci, thereby influencing DNA methylation and histone modifications (Pathania et al., 2021). For example, ncRNAs can recruit DNA methyltransferases to target sites, leading to gene silencing, or they can interact with histone modifiers to alter chromatin structure and gene expression (Palicelli et al., 2021). The regulatory roles of ncRNAs are essential for various cellular processes, including development, differentiation, and response to environmental stimuli (Bure et al., 2022).

4.4 Crosstalk between epigenetic mechanisms

The crosstalk between different epigenetic mechanisms, such as DNA methylation, histone modifications, and RNA modifications, adds an additional layer of complexity to gene regulation. For instance, N6-methyladenosine (m6A) RNA methylation has been shown to interact with histone and DNA modifications, influencing RNA stability and translation efficiency (Kan et al., 2021; Zhao et al., 2021). This intricate interplay ensures coordinated regulation of gene expression, allowing cells to respond dynamically to internal and external cues. Understanding these interactions is crucial for unraveling the complexities of epigenetic regulation and its impact on gene expression in Bt (Song et al., 2021).

5 Case Study: Epigenetic Regulation of *Cry* Genes in Bt

Bacillus thuringiensis (Bt) is a bacterium known for its insecticidal properties, primarily due to the production of Cry proteins. These proteins are widely used in biopesticides and transgenic crops to control insect pests. However, the effectiveness of Bt products is increasingly threatened by the evolution of resistance in target insect populations. Understanding the molecular mechanisms, including epigenetic factors, that regulate *Cry* gene expression and contribute to resistance is crucial for the sustainable use of Bt-based technologies.

5.1 Analysis of epigenetic factors affecting *Cry* gene expression

Recent studies have highlighted the role of various transcription factors and signaling pathways in the regulation of *Cry* gene expression. For instance, the MAPK signaling pathway has been shown to influence the expression of Cry1Ac receptor genes. In *Plutella xylostella*, the transcription factor PxGATAd, activated by the MAPK pathway, directly interacts with a specific GATA-like cis-response element in the promoter region of the *PxmALP* gene, a Cry1Ac receptor. Mutations in this cis-acting element can repress transcriptional activity, thereby reducing susceptibility to Cry1Ac toxin (Figure 2) (Guo et al., 2022). Similarly, the transcription factor PxJun, also activated by the MAPK pathway, has been found to repress the expression of the *PxABCB1* gene, another Cry1Ac receptor, contributing to resistance (Qin et al., 2021).

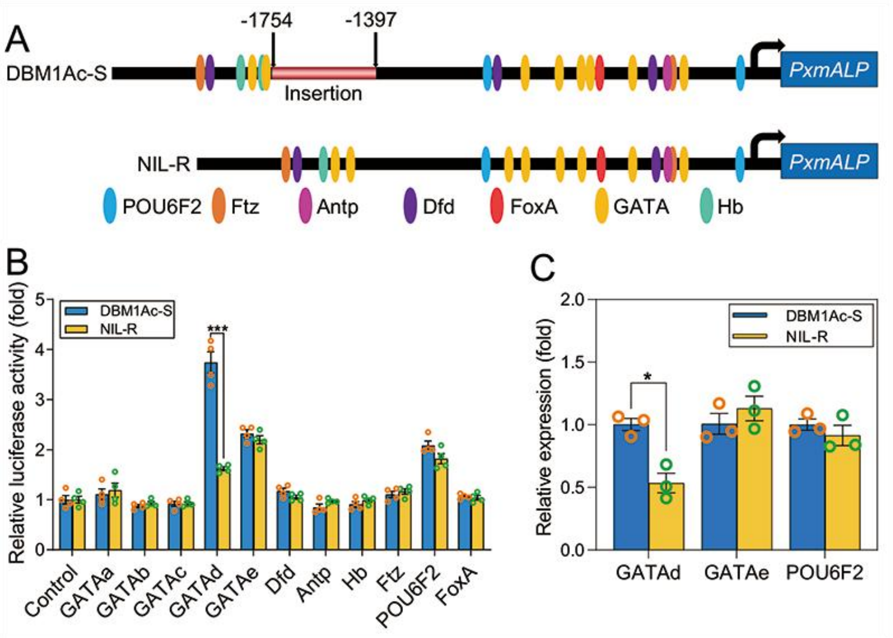

Figure 2 Role of different TFs on the transcriptional activation of the *PxmALP* promoter (Adopted from Guo et al., 2022)

Image caption: (A) Schematic illustration of the putative TF CREs located in the promoter region of *PxmALP*. Different CREs are indicated by colored ellipses. (B) Analysis of various TFs on the activity of the *PxmALP* promoter. The values shown are the means and the corresponding SEM values. The empty vector pAc5.1/V5-His B was used as control and standardized the value as 1-fold. Four independent transfections were conducted for each pair of plasmids. Holm-Sidak's test was used for statistical analysis (***$p < 0.001$). (C) The expression of *PxGATAd*, *PxGATAe* and *PxPOU6F2* was analyzed in the midgut tissue from larvae of DBM1Ac-S and NIL-R strains. The values are presented as mean of relative expression values ± the SEM (*$p < 0.05$, Holm-Sidak's test, $n = 3$) (Adopted from Guo et al., 2022)

Figure 2 illustrates the role of the transcription factor PxGATAd in regulating the expression of the *PxmALP* gene. Figure 2A shows the prediction of multiple potential cis-acting elements in the promoter region. The luciferase reporter assay in Figure 2B indicates that PxGATAd significantly enhances the activity of the *PxmALP* promoter in the Cry1Ac-susceptible strain, while the effect is weaker in the resistant strain. The qPCR results in Figure 2C further confirm that the expression level of PxGATAd is significantly higher in the susceptible strain compared to the resistant strain. Overall, Figure 2 reveals that PxGATAd is a key regulatory factor for *PxmALP* gene expression, and its expression is suppressed in the resistant strain, leading to the diamondback moth's resistance to Cry1Ac toxin.

Moreover, microRNAs (miRNAs) have been implicated in the post-transcriptional regulation of Cry receptor genes. For example, miR-998-3p targets the coding sequence of the ABCC2 gene, a known Cry1Ac receptor, and down-regulates its expression in several lepidopteran pests, thereby increasing resistance to Cry1Ac toxin (Zhu et al., 2019).

5.2 Impact on the insecticidal properties of Bt

The epigenetic regulation of Cry gene expression has significant implications for the insecticidal properties of Bt. The down-regulation or mutation of Cry receptor genes, mediated by transcription factors and miRNAs, can lead to reduced binding of Cry toxins to midgut cells in insects, thereby decreasing their susceptibility to these toxins. For instance, the reduced expression of PxmALP and PxABCB1 in P. xylostella due to the action of PxGATAd and PxJun, respectively, results in higher levels of resistance to Cry1Ac toxin (Qin et al., 2021; Guo et al., 2022). Similarly, the down-regulation of ABCC2 by miR-998-3p in various lepidopteran pests also contributes to increased resistance (Zhu et al., 2019).

6 Environmental Factors Influencing Epigenetic Modifications in Bt

6.1 Role of environmental stressors in epigenetic changes

Environmental stressors such as drought, cold, salinity, and heat can induce significant epigenetic changes in organisms, including Bt (*Bacillus thuringiensis*). These stressors can lead to alterations in DNA methylation, histone modifications, and the expression of non-coding RNAs, which in turn affect gene expression and phenotypic traits. For instance, plants exposed to abiotic stresses adopt epigenetic changes to cope with these conditions, which can be temporary or stable and inheritable (Chang et al., 2019; Akhter et al., 2021). Similarly, in Bt, environmental stressors may trigger epigenetic modifications that help the bacteria adapt to and survive in challenging environments.

6.2 Interaction between Bt and host plants

The interaction between Bt and host plants is another critical factor influencing epigenetic modifications. Plants and their associated microorganisms, including Bt, are exposed to fluctuating environmental conditions that necessitate adaptive responses. Epigenetic regulation plays a significant role in this interaction by modulating gene expression patterns in response to environmental cues. For example, plants exhibit phenotypic plasticity largely determined by epigenetic regulation, which allows them to adapt to varying environmental conditions (Brukhin and Albertini, 2021). This interaction can lead to epigenetic changes in Bt, potentially affecting its virulence and effectiveness as a biocontrol agent.

6.3 Influence of external chemicals and pollutants

External chemicals and pollutants are known to induce epigenetic changes in various organisms, including Bt. Pollutants such as heavy metals, endocrine disruptors, and other environmental contaminants can cause oxidative stress and DNA damage, leading to epigenetic modifications like DNA methylation and histone modifications (Sharavanan et al., 2019; Han and Huang, 2021). These changes can result in gene dysregulation and altered physiological responses. For instance, exposure to pollutants has been shown to induce epigenetic changes that affect reproductive toxicity and other health outcomes in different species (Sharavanan et al., 2019). In Bt, similar mechanisms may be at play, where exposure to external chemicals and pollutants could lead to epigenetic modifications that impact its gene expression and overall functionality.

7 Implications of Epigenetic Modifications for Bt Application

7.1 Epigenetic modifications and Bt efficacy

Epigenetic modifications, such as DNA methylation and histone modifications, play a crucial role in regulating gene expression without altering the underlying DNA sequence. These modifications can significantly impact the efficacy of *Bacillus thuringiensis* (Bt) by influencing the expression of genes involved in toxin production and resistance mechanisms. For instance, the reversible nature of epigenetic changes, such as DNA methylation and histone acetylation, can modulate the expression of genes that are critical for Bt's insecticidal activity (Han et al., 2019; Jin et al., 2021). Understanding these modifications can help in optimizing Bt strains for enhanced efficacy and stability in various environmental conditions.

7.2 Potential for epigenetic modulation in Bt-based products

The potential for epigenetic modulation in Bt-based products is vast. By targeting specific epigenetic regulators, it is possible to enhance the expression of beneficial genes while suppressing those that may confer resistance or reduce efficacy. For example, the use of CRISPR/Cas-based systems to edit the epigenome has shown promise in other fields and could be adapted for Bt applications (Rittiner et al., 2022). Additionally, small molecule inhibitors that target epigenetic enzymes, such as DNA methyltransferases and histone deacetylases, could be used to fine-tune gene expression in Bt, thereby improving its insecticidal properties and reducing the likelihood of resistance development (Cheng et al., 2019; Han et al., 2019; Jin et al., 2021).

7.3 Future directions in Bt epigenetics research

Future research in Bt epigenetics should focus on several key areas. Comprehensive mapping of the epigenetic landscape in Bt strains will provide insights into how these modifications influence gene expression and toxin production. The development of advanced tools for precise epigenetic editing, such as CRISPR/Cas systems, will enable targeted modifications to enhance Bt efficacy (Cheng et al., 2019; Rittiner et al., 2022). Exploring the interplay between epigenetic modifications and environmental factors will help in understanding how external conditions influence Bt performance. Furthermore, integrating epigenetic approaches with traditional genetic engineering techniques could lead to the development of next-generation Bt products with superior insecticidal properties and reduced resistance potential (Han et al., 2019; Jin et al., 2021).

8 Concluding Remarks

Epigenetic modifications play a crucial role in the regulation of gene expression in *Bacillus thuringiensis* (Bt) and its interaction with insect pests. Studies have shown that down-regulation of specific genes, such as the P-glycoprotein gene *PxABCB1*, is associated with resistance to Bt toxins in pests like *Plutella xylostella*. Whole genome sequencing of various Bt strains has revealed the presence of multiple pesticidal protein genes, which contribute to their insecticidal properties. Additionally, genetic modifications, such as the deletion of the *leuB* gene, have been shown to enhance the stability and efficacy of Bt biopesticides under field conditions. The interaction between Bt infection, host immunity, and gut microbiota also significantly influences the effectiveness of Bt as a biocontrol agent.

Understanding the epigenetic modifications in Bt is vital for advancing agricultural biotechnology. These modifications can influence the expression of genes responsible for toxin production and resistance mechanisms in pests. For instance, the down-regulation of *PxABCB1* in Cry1Ac-resistant *P. xylostella* highlights the importance of gene expression regulation in developing resistance management strategies. Moreover, the identification of multiple pesticidal protein genes through genomic studies provides a valuable resource for developing new Bt strains with enhanced insecticidal properties. Genetic engineering approaches, such as CRISPR-Cas9-mediated gene editing, offer promising tools for optimizing Bt strains for better performance and stability in agricultural applications. By leveraging these insights, researchers can develop more effective and sustainable biopesticides, reducing the reliance on chemical pesticides and mitigating the impact of pest resistance.

The study of epigenetic modifications in Bt and their impact on gene expression is a rapidly evolving field with significant implications for agricultural biotechnology. The integration of genomic, transcriptomic, and proteomic data provides a comprehensive understanding of the molecular mechanisms underlying Bt's insecticidal activity and pest resistance. Continued research in this area will enable the development of innovative strategies to enhance the efficacy and stability of Bt biopesticides, ultimately contributing to sustainable pest management and improved crop protection. As we advance our knowledge of epigenetic regulation in Bt, it is essential to translate these findings into practical applications that benefit farmers and the environment.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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